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Reproductive biology of pequira *Bryconamericus stramineus* (Eigenmann, 1908) in Funil Reservoir-MG, Brazil

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(With 5 figures)

Abstract

In order to evaluate aspects of reproductive biology of the “pequira” (*Bryconamericus stramineus*) in the elevator of the Funil Dam - MG, fish capture was carried out from November 2008 to January 2009 and 317 individuals were collected. The mean standard length (SL) of the population was 4.96 cm and mean weight 1.80 g. The females had SL of 5.0 cm, while males had a SL of 4.6 cm. A sex ratio of 2.20: 1 (females: male) was observed. Our results show that 73% of the individuals analyzed were considered adults. The species presented low fecundity, mean of 470.9 oocytes per female and a mean diameter of 221.08 µm, with an increase in oocyte diameter over the evaluation period. The length of the first gonadal maturation was estimated at 5.0 cm. The results obtained in this work suggest that the reproductive cycle of the species occurs in the analyzed period. Although this species does not have migratory reproductive habits, the presence of adults in the reproductive stage was observed in the transposition area, which suggests a search for new environments for spawning.

Keywords: sex ratio, age structure, gonadal maturation, reproductive period.

Biologia reprodutiva da Pequira *Bryconamericus stramineus* (Eigenmann, 1908) no reservatório de Funil-MG, Brasil

Resumo

Com o objetivo de avaliar aspectos da biologia reprodutiva da pequira *Bryconamericus stramineus* no elevador da represa do Funil - MG foram realizadas capturas entre os meses de novembro de 2008 a janeiro de 2009, sendo coletados 317 indivíduos. O comprimento padrão (CP) médio da população foi de 4,96 cm e peso médio de 1,80 g. As fêmeas apresentaram CP de 5,0 cm, enquanto que os machos obtiveram um CP de 4,6 cm. Foi observada proporção sexual de 2,20:1(fêmeas:macho). Dos indivíduos analisados, 73% foram considerados adultos. A espécie apresentou baixa fecundidade, com média de 470,9 ovócitos por fêmea e diâmetro médio de 221,08 µm, ocorrendo aumento no diâmetro de ovócitos com o decorrer do tempo no período avaliado. O comprimento de primeira maturação gonadal foi estimado em 5,0 cm. Os resultados obtidos neste trabalho sugerem que o ciclo reprodutivo da espécie ocorre no período analisado. Embora esta espécie não tenha hábitos reprodutivos migratórios, a presença de adultos no estágio reprodutivo foi observada na área de transposição, o que sugere a busca de novos ambientes para a reprodução.

Palavras Chave: proporção sexual, estrutura etária, maturação gonadal, período reprodutivo.

1. Introduction

Pequira (*Bryconamericus stramineus* Eigenmann, 1908) is a South America native fish specie, with non-migratory habit and diurnal activity (Bizzotto et al., 2009), found in La Plata, and São Francisco rivers (Planquette et al., 1996; Lampert et al., 2007). Despite its small size, about 76 mm in length (Ringuelet et al., 1967), pequira is consumed as a protein source by the riverside population and is also important as food for other fish species.

Few studies have been conducted concerning to *Bryconamericus* species reproduction (Lampert et al., 2007). Some examples were found by Godoy (1975), Nakatani (2001), and Winemiller (1989) for *Bryconamericus beta* and also by Lampert et al. (2004), who studied *Bryconamericus iheringii* in Vacacaí River in Rio Grande do Sul State (Brazil). Lampert et al. (2007) observed that *B. stramineus* has split spawning and that the breeding season occurs between September and December, having a lower peak in February in the Ibicuí River, a tributary of Uruguay River Basin (Rio Grande do Sul, Brazil).

Knowledge of reproductive tactics is fundamental to comprehend the species life cycle strategies, which are crucial to direct administration, management, and preservation measures from certain human actions, such as fishing, pollution, and elimination of reproductive areas by constructing dams and destroying marginal vegetation (Vazzoler and Menezes, 1992). During fish annual reproductive cycle, remarkable changes can be observed in their gonads. The gonadosomatic index is an important parameter for evaluating this fish's phase, expressed as the gonads development, being an accurate indicator of ovaries functional state (Wooton, 1999).

The fish gonads maturation process and reproduction initiation are influenced from ingested food or deposited energy reserves in different body parts (Vazzoler, 1996). Therefore, by knowing the species maturation curve, it can be correlated to the individual nutritional status and to the ability to mobilize these reserves (Bazzoli et al., 1997). During reproduction cycle, fish stop feeding and endogenous nutrients are converted into energy to the gonads, and hence there is weight and energy reserves reduction (Maddock and Burton, 1998). This work aimed to know the reproductive biology of *B. stramineus* specimen captured in the Funil Hydroelectric Power Plant, Lavras-MG, from November 2008 to January 2009.

2. Materials and Methods

The study was conducted at Funil Hydroelectric Power Plant (Funil HPP), that is located at 21° 08'38 S 45° 02'11" W at the top of the Grande River, between Furnas reservoir and Itutinga dam. The fish transposition system (FTS) implanted in Funil HPP is elevator type with fish air capture and transport. This is equipped with four main parts: input channel, mechanic elevator, leakage channel, and auxiliary water system. The input channel is about 2.40 × 26 meters and is located on the left bank of the leakage canal. The average temperature and rainfall respectively observed in the Funil dam area was 21.8° C and 0.32 mm^3 in November/2008, 22.0 °C and 0.67 mm^3 in December/2008, and 22.7° C and 0.52 mm^3 in January/2009.

A total of 317 animals were captured. The captures were made directly in the transposition tank at the exit of the elevator and the fish were captured with a handle. The animals were captured during the daytime period in the second week of each of the evaluated months, November and December of 2008, and January of 2009. After capture, the animals were anesthetized with eugenol and later fixed in 10% formalin solution, and transported for the Animal Physiology and Pharmacology Laboratory, Veterinary Medicine Department at the Federal University of Lavras-UFLA. For each specimen, measurements of standard length (SL) were recorded in cm and fish total weight (TW), in grams.

Through a ventral incision, all specimens were opened, and the viscera were removed. The gonads were examined macroscopically with a stereomicroscope to determine the sex and maturity stages.

The sex ratios (SR) were determined in the experimental period, by month and by size class, through the expression: SR = number of females/number of males. Differences in sex ratio were tested for each study month, by the chi-square (x^2).

The gonads were classified macroscopically by size in relation to the abdominal cavity, color, blood vessels presence, size, and appearance. For females, an adaptation of the rating scale described by Vazzoler (1996) was used, comprising four stages: immature (F1), when the ovaries size is reduced, occupying the coelomic cavity less than 1/3, filamentous, translucent, without vascularization signs, and the oocytes are not observed with the naked eye; maturing (F2), when the ovaries occupy 1/3 to 2/3 of the coelomic cavity, with intense vascularization and oocytes are opaque, small, and medium-sized; mature (F3), when ovaries occupy almost all the coelomic cavity, having turgid, large, opaque, and/or translucent oocytes, whose frequency varies with the maturation progress; and exhausted (F4), for ovaries in varying degrees of laxity, membrane distended and hemorrhagic appearance, occupying less than 1/3 of the coelomic cavity, with few oocytes.

For males, a range of four stages was followed (Lampert et al., 2007), immature (M1), when testicles were shown to be filamentous, translucent, and without vascularization; in maturation (M2), when larger testicles than in phase before become opaque and reddish white, and vascularization is most noticeable; mature (M3), when testicles reach considerable size, presenting opaque, reddish white coloration with prominent vascularization; and exhausted (M4), when the testicles are completely limp with the elimination of sperm, presenting hemorrhagic aspect due to blood vessels disruption.

The ovaries in the mature stage were removed, weighed on a digital analytical balance (0.01 g), fixed in alcohol solution of 70%, identified and stored individually in vials until analysis. The first gonadal maturation size, considered as one in which 50% of the fish are able to reproduce (L_{50}), was estimated from the regression between the proportion of mature individuals and classes with 1 mm interval. All individuals who had immature gonads and standard length equal to or less than the value obtained for the L_{50} were considered young.

The gonadosomatic index (GSI), representing the percentage contribution of the gonads mass in relation to total body mass, was calculated for females, using the expression (McAdam et al., 1999): GSI = (GW/TW) × 100, in which GW = gonad weight and TW = fish total weight. The coelomic fat index (CFI) was assessed visually. Values from

1 to 4 were assigned, each value corresponding to a coelomic cavity percentage filled with fat: 1- from 0 to 25%, 2- from 25 to 50%, 3 - from 50 to 75%, and 4 from 75 to 100%. The stomachic repletion stages (SRS) were determined by visual quantitative evaluation of stomach contents, being classified: SRS I is an empty stomach; SRS II, semi-full stomach; and SRS III, full stomach.

The stomach repletion index was analyzed, using the expression established by Santos (1978): $SRI = (SW/WWG) \times 100$ and $aSRI = \Sigma SRI/N$, in which SRI = stomachic repletion index, SW = stomach weight, WWG = fish weight without the gonad, aSRI = average stomachic repletion index, and N = number of individuals.

K1 and K2 condition factors of Fulton were estimated for each individual, using Le Cren formula (Le Cren, 1951): $K1 = TW/SL^b$ and $K2 = WWG/SL^b$, in which TW = total fish weight, WWG = fish weight without gonad, SL = individual standard length and b = constant equal to 3.

The fecundity was estimated by withdrawing an anterior sample, middle, and posterior of each stored mature ovary, which was weighed and examined in an optical microscope and the present oocytes were counted. As oocytes were observed in two different sizes, their counting was performed for each of these groups. From the oocytes total number in the sample and oocytes weight by the gonad total weight, total oocytes number in each female was estimated and then the *B. stramineus* average fertility was calculated.

The oocytes diameter was analyzed with the aid of an optical microscope, being 10 oocytes measured from each mature ovary, 5 of each size group, on two axes, horizontal and vertical, because they were not spherical. The egg diameter final value was determined by calculating the mean of found diameter and then converted to micrometers.

Differences in sex ratio were tested using the chi-square test (x^2) performed in the Statistica 6.0 software

3. Results

Of the total 317 *B. stramineus* individuals, 124, 99 and 94 were captured in November/08, December/08 and January/09, respectively.

Fish average standard length (SL) was 4.96 cm; the smallest examined individual during the study period measured 3.7 cm and the biggest 6.0 cm. *B. stramineus* females had higher SL than males, 5.00 ± 0.43 cm and 4.67 ± 0.39 cm, respectively. There were no significant differences between the individual's distribution by size class, over the study months.

However, significant differences were observed in sex ratio throughout the study, with respect to 2.20 females per male, and for the months of December and January singly (Table 1). The tendency was to increase the females' proportion, with the size classes increasing (Table 1).

Macroscopically, there were changes in ovaries thickness, volume, and staining during the different *B. stramineus* gonadal maturation stages. The observed staining in the *B. stramineus* ovaries was translucent in the immature stage, changing gradually as maturation occurred from shades of yellow-orangish to orange-reddish in the advanced maturity stage, occupying almost the whole coelomic cavity.

Of the 218 analyzed females, 109 (50%) were in the immature stage (F1), 66 (30.2%) maturing (F2), and 43 (19.8%) mature (F3). There were no spawning females (F4) during the study period.

About female gonadal maturation stages in each length class, it was observed that number of females in the maturing and mature stages increases and the number of immature ones falls with the SL increasing (Figure 1).

Some changes were observed in thickness, volume, and testicles staining during the *B. stramineus* reproductive cycle's different phases. The observed staining in the *B. stramineus* testicles was translucent white in the immature stage, changing into milky white in the advanced maturation stage.

Among 99 studied males, 15 (15.1%) were in the immature stage (M1), 39 (39.4%) in maturation (M2), and 45 (45.5%) mature (M3). There were no exhausted males (M4) in the study period.

With specimens' standard length increasing, it was observed decrease in the number of immature and maturing males with an increase of mature males, and the last size class, few maturing individuals were observed with a predominance of mature fish (Figure 2).

Table 1. *Bryconamericus stramineus* sex ratio in each length class.

Length classes		Females	Males Females/ Males	Sex ratio
1°	3.7 ⊢ 4.2	4	2	2:1
2°	4.2 ⊢ 4.7	38	23	1.65:1
3°	4.7 ⊢ 5.2	92	48	1.92:1*
4°	5.2 ⊢ 5.7	63	22	2.86:1*
5°	5.7 ⊢ 6.0	21	4	5.25:1*

*values differ statistically according to the x^2 test $p < 0.001$.

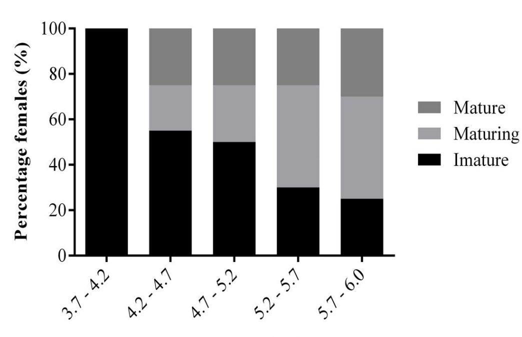

Figure 1. Percentage of *B. stramineus* females in each length class at different stages of gonadal maturation, captured between November 2008 and January 2009.

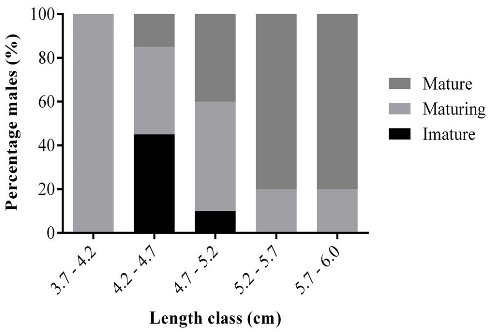

Figure 2. Percentage of *B. stramineus* males in each length class at different stages of gonadal maturation, captured between November 2008 and January 2009.

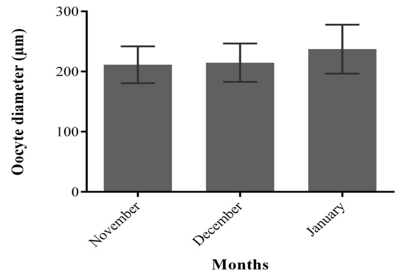

Figure 3. Mean oocyte diameter of *B. stramineus* in each collection month.

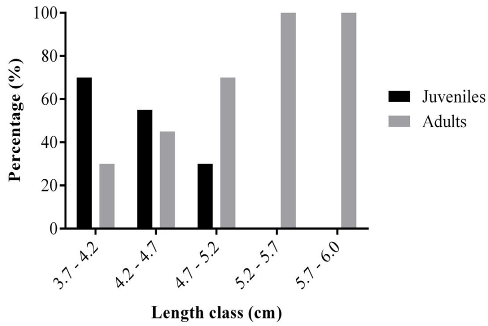

Figure 4. Percentage of *B. stramineus* juveniles and adults in each length class, captured between November 2008 and January 2009.

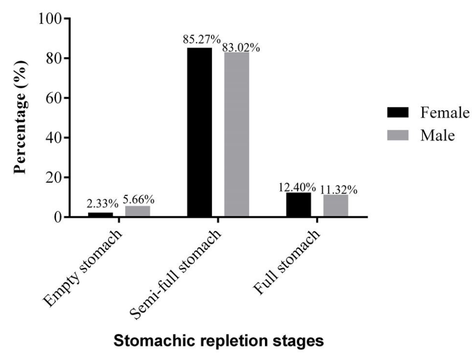

Figure 5. Percentage of *B. stramineus* individuals in each class of stomach repletion stage, captured between November 2008 and January 2009.

During the microscopic ovaries analysis, two distinct oocytes classes were observed, one of small developing oocytes and one of large oocytes in vitellogenesis. The average total fertility rate for females was 470.9 ± 270.9 oocytes during the period studied, with an average of 81.85% of large oocytes and 18.15% small.

Over the study months, an increase in the oocytes number was observed. In November/08, December/08 and January/09 there was an average of 405 ± 245.3, 488 ± 289.2, and 520 ± 302.3 oocytes per female, respectively.

The average oocytes diameter was 221.08 ± 35.34 µm, having an increasing over the same sampling period (Figure 3).

The $L_{50,}$ length at which 50% of individuals able to reproductive, was determined to be 5.0 cm for females and 4.8 cm for males. Thus, L_{50} population was considered equal for both, females and males. The percentage of young and adults of *B. stramineus* in each length class is shown in Figure 4.

The gonadosomatic index (GSI), which represents the percentage contribution of the gonads weight in relation to total body weight, presented to the female value of 5.05. When analyzed monthly, the GSI had values of 5.64, 4.21, and 5.41 for the months of November/08, December/08 and January/09, respectively. Most of the evaluated males and females are found in classes two and three of coelomic fat index (CFI) (Figure 5), observing an inverse relationship between the stage of gonadal maturation and this factor, more evident for females.

Most of the examined samples was in stomachic repletion intermediate stage (SRS) (Figure 5), having no differences in this parameter among the months, between genders, or among maturation stages. There were no significant differences between genders for each stage of stomach repletion (SRS).

Average SRI value for the experimental period was 1.12, with little variation among males and females, and among different maturation stages (Table 2). There were no differences between K1 and K2 values, among the collection months, and between the sexes, ranging between 0.014 and 0.015.

Table 2. Stomachic repletion index (SRI), stomach average weight (SW) and fish average weight without the gonad (WWG) of *Bryconamericus stramineus* in sexual maturation stage.

	Maturity stage	SRI	SW	WWG
Females	Immature	1.12	0.02	1.73
	Maturing	1.09	0.02	2.01
	Mature	1.07	0.02	1.77
	Average	1.10	0.02	1.83
Males	Immature	0.96	0.01	1.39
	Maturing	1.29	0.02	1.59
	Mature	1.17	0.02	1.85
	Average	1.18	0.02	1.66

4. Discussion

The shorter length found among the analyzed samples is higher than the average found by Lourenço et al. (2008b) for the same species, in which the average SL was 3.12 cm. The difference among found lengths may be justified by the fact that the collection has been conducted at the pass system top and, to enter into the fish lift, the fish must overcome a gap between the downstream river and the lift channel entrance, and may restrict the smaller fish entry that can not overcome it.

Lourenço et al. (2008b) studying *B. stramineus* population in the upper Paraná river observed a polymodal pattern, which may indicate repeated reproductive episodes throughout the year, and therefore the presence of several cohorts in the population.

The occurrence of a greater females proportion compared to males in a fish population is also reported by Mazzoni and Silva (2006) in *Bryconamericus microcephalus*, having found a sex ratio of 2.20:1 (female:male). These results are consistent in accordance with that expected for Characidae, in which although it is expected to find the sex ratio of 1:1, usually a greater females proportion is observed (Hermes-Silva et al., 2008). The sex ratio in a population can vary by several factors, which may be environmental or physiological, or a combination of two factors, such as mortality or differential growth between the sexes (Vazzoler, 1996), the population food supply (Nikolsky, 1969), and predation (Raposo and Gurgel, 2001). Through the results about the population sex ratio, one can deduce whether the growth is occurring or not. In oligotrophic reservoirs and rivers there is males' predominance; females predominate when the available food is abundant (Raposo and Gurgel, 2001). Thus, a higher females' frequency means a population's response to favorable conditions provided by the environment.

The presence of young fish in the lift may be due to the attraction of this waterfall at its entrance or these animals may be carrying out a migration of the regional colonization to the dam's upstream and not a reproductive migration. Increasing regional temperature and rainfall during the study period may have influenced the gonads maturation, as these factors are environmental variables that influence individuals, so that conditions in the spawning season are conducive to offspring survival and growth (Lourenço et al., 2015, Vazzoler, 1996).

A large *B. stramineus* female portion was found in Ibicuí River between October and November (Lampert et al., 2007). Godoy (1975), also studying *B. stramineus*, in Mogi Guaçu river, observed the period between September and January as reproduction species season. Other spawning species such as electric fish (*Eigenmannia trilineata*) have a reproductive peak during the migration of migratory fish, which are influenced by changes in abiotic factors (Giora and Fialho, 2009).

Although Bizzotto et al. (2009) consider the species as a non-migratory species, in their study they found a peak rise in pequira by a transposition system such steps in the months of December and January and falling sharply in the coming months. These results demonstrate that during the spawning pequira population migration occurs upstream. Some fish species can perform migrations for food (Fernandez et al., 2007), not being necessarily for reproduction.

There were no spawning females during the study period, which allows us to suggest that the collection site is used by the species as a migratory route, looking for the spawning grounds upstream. However, this do not mean that the species reproduces after this route. This is due to common occurrence in oocytes resorption fish if the environmental conditions and/or animal physiological did not present themselves favorable for reproduction during spawning. This process can occur before and after spawning. Usually it occurs before spawning for oocytes that have not reached maturity and after spawning for those who ceased to be eliminated, in natural or artificial environments (Ganeco et al., 2001).

The *B. stramineus* average fertility is lower than in other family members that have external fertilization. A lower average fertility than that found in this work was reported by Lampert et al. (2007) for *B. stramineus* in Ibicuí River, where they observed a mean fecundity of 371.3 ± 244.6 oocytes.

Species that have low fertility often have other feature as parental care, internal fertilization, or multiple spawning, as these strategies can offer to eggs and larvae higher fertilization possibilities and/or survival (Vazzoler and Menezes, 1992). However, there is no known parental care feature or any other particular reproductive characteristic that increases to eggs and larvae survival's chances, allowing oocytes production relatively low for this *Bryconamericus* species (Lampert et al., 2007). These changes are reflected in different fecundity among populations and species (Galvani and Coleman, 1998).

The length at first maturity can vary among the same species populations in environments with physical characteristics and distinct hydrological scheme (Lourenço et al., 2008a). L_{50} lower value (4.2 cm) to this species is reported for *B. microcephalus* (Mazzoni and Silva, 2006).

According to Guraya (1986), increasing the ovaries weight of many fish in relation to total body weight of 1% or less until 20% occurs in the period before spawning, due to mainly the reserve for embryos nutrients accumulation, the vitelo. Lampert et al. (2007) in *B. stramineus* observed that the GSI values in both sexes began to increase slightly in September, reaching the highest values in October and November. They noted the GSI value for females of 5.19 ± 1.39 in November/01, 3.20 ± 2.72 in December/01, and 1.44 ± 0.97 in January/02. So, they characterized this period as the species reproductive season, when the regional temperature was the highest. GSI decreased after this period. However, in the present study, there was a less intense reduction of GSI values in the same evaluation periods (November/08 = 5.64, December/08 = 4.21 and January/09 = 5.41.

The CFI behavior in relation to ovarian development suggests involvement of this fat in the oocyte maturation process in *B. stramineus* female. During the gonadal maturation, the coelomic consumption is intense (Barreto et al., 1998). For males, the gonadal maturation processes and gametes release are somewhat less complex, consisting primarily of cell division, while for females more complex processes occur, such as vitellogenin production. This fact may have contributed to none observation done in this study, in relation to fat reserves mobilization for testicular development.

In B. stramineus any change in feeding activity during this period did not occur. A similar result was found by Mazzoni and Rezende (2009), who did not observe differences in the feeding activity of B. microcephalus. It could therefore to associate the variation observed in SRI to the gradual mechanical compression exerted by the gonad volume increasing in the coelomic cavity. This fact also explains the variation occurrence only in females, because the ovaries have wide variation in weight and volume, while the testicles changing is more discreet.

The condition factor can be influenced, among other factors, by changing in gonads weight, stomach and coelomic fat. Therefore, the closeness among the observed values in all tests in both K1 and K2 may be a reflection of the high CFI, SRS, and SRI found during the studied species. There is an energetic reserve transfer that accumulated in the viscera, muscle, and/or liver to meet the gonads development, which culminated with the spawn, reflecting changes in condition factor (Vazzoler, 1996). However, in this study such changes were not observed.

The value of K1 and K2 condition factor were very close, indicating that in this case there would not be mobilization of animal energetic reserves to the gonads maturation in the studied species. However, when a female CFI is analyzed, it is observed that a visceral fat decrease occurs with gonadal development.

In conclusion, we can say that the species reproduces during the analyzed period; the females have the mobility of their coelomic fat reserves to the gonads during gonadal maturation, having low fecundity. In addition, the results demonstrate that although this species does not have migratory reproductive habits, the presence of adults in the reproductive stage was observed in the transposition area, which suggests a search for new environments for spawning.

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